Survival of hepatitis A and E viruses in soil samples

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Abstract

Survival of hepatitis A virus (HAV) and hepatitis E virus (HEV) in soil samples spiked with respective viruses was analysed using real-time PCR. Virus-spiked soil samples were incubated at environmental temperature (ET) and 37°C and processed weekly. Both HAV and HEV were less stable at fluctuating ET than at 37°C. Of the 403 soil samples collected in the vicinity of Mutha river, India, 19.1% and 4.9% were found to be contaminated with HAV and HEV, respectively.

Keywords: Environment, hepatitis A virus, hepatitis E virus, soil, survival

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Hepatitis A (HAV) and hepatitis E (HEV) viruses are the predominant causes of enterically transmitted hepatitis. Though both viruses usually lead to self-limiting disease, fulminant hepatic failure with fatal outcome occurs in a small proportion of patients. Sewage/faecal contamination of water remains the major cause of transmission of these viruses [1–3]. Understanding of the prevalence and persistence of enteric viruses in environmental samples is an important issue for the effective control of these infections. Soil from the areas with contaminated water is a likely vehicle for the transmission of enteric viruses. Reo and polio viruses survive in soil samples, increasing the risk of transmission [4]. So far, studies on the survival of HAV or HEV in soil have not been reported. This study addresses the survival of these viruses in soil at various temperatures and at different time-points.

A total of 0.5 gm soil was spiked with 1.23×10^8 RNA copies of HAV (genotype IIIA) and 6.47×10^7 RNA copies of HEV (genotype 1) present in the faecal samples of hepatitis A and E patients, respectively. The test and control (receiving only phosphate buffered saline) soil samples in transparent collection boxes were incubated at environmental temperature (ET) or 37°C and processed weekly for RNA extraction, followed by real-time PCR. The samples were treated with 7.5 M potassium acetate in order to remove humic acid, a strong PCR inhibitor present in the soil. RNA was extracted using hexadecyltrimethylammonium bromide (CTAB) extraction buffer according to the protocol of Griffiths *et al.* [5] and the QIA-amp Viral RNA Minikit method (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Real-time PCR for HAV [6] and HEV [7] was carried out, detection limits being 100 and 10 RNA copies, respectively. Statistical analysis was performed using the chi-squared test for the comparison of proportions.

During January to December 2008, a total of 403 soil samples were collected twice a month from approximately 3-cm depth along four different points (P1–P4, Fig. 1a) of the Mutha river, Pune, India. No outbreak of waterborne viral disease was recorded in Pune city during the period of sample collection and to date. As negative controls, 57 samples were collected from a well-protected Pashan Lake not contaminated by sewage. Soil samples were tested for pH, salt concentration, relative humidity and UV-VIS fluence/flux according to IS 2720 (Part 26) 1987, IS 2720 (Part 21) 1977, ASTM – D1412 and ASTM standard E 169–04 methods, respectively.

Optimization of RNA Extraction Procedure

Extraction with CTAB yielded 1–3 logs lower RNA yields for HAV and HEV, respectively, and therefore the QIA-amp method was used for all the experiments. Traces of phenol or chloroform used during the CTAB method may have led to the inhibition of PCR reactions.

Treatment of Soil Samples with Potassium Acetate

The presence of humic acid in soil samples inhibits PCR reactions even at concentrations as low as 10 ng per

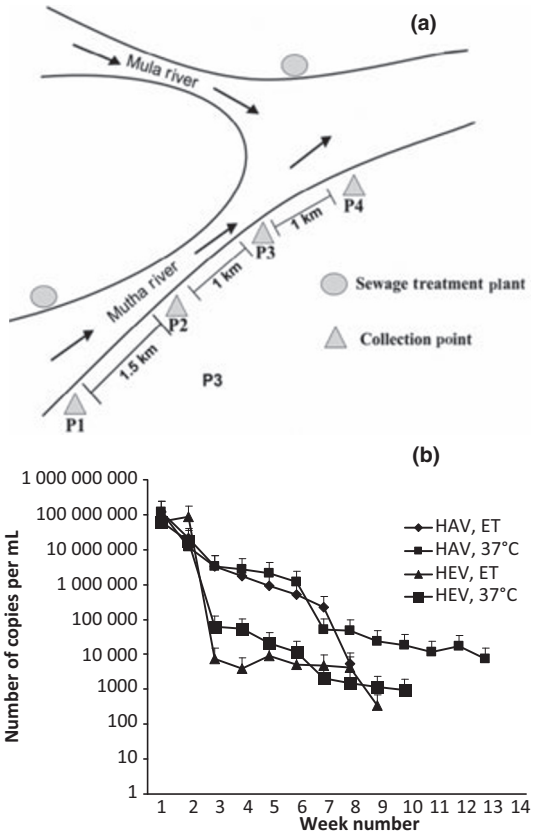

FIG. 1. (a) Location of sample collection points from the Mutha river, Pune. (b) Survival of HAV and HEV in spiked soil samples at environmental temperature and 37°C.

reaction [8], which can be reversed by treating the samples with potassium acetate [9]. However, such a reversal was not observed when we treated the virus-spiked soil samples with 7.5 M potassium acetate.

Soil Parameters

The values obtained for the four parameters examined over a period of 1 year were: pH (7.27–8.19), relative humidity (43–67%), UV-VIS fluence/flux (26.43–31.94 mmol) and salts (35–273 p.p.m.). No relationship of soil parameters with viral RNA positivity was observed (data not shown).

Survival of HAV and HEV in the Spiked Soil Samples

HAV could survive for 8 weeks at ET and 13 weeks at 37°C (Fig. 1b) while HEV survived for 9 weeks at ET and 10 weeks at 37°C (maximum temperature range, 30.5°C – 37.4°C; minimum temperature range, 11.9°C – 17.1°C) (Table 1). Thus, survival time for both the viruses was comparable at ET. At 37°C HAV was relatively more stable than HEV. However, the reduction pattern differed; HAV declined steadily over time whereas a sudden decrease was noted for HEV at the third week, reaching a plateau during weeks 3–8. These results reflect stability of specific viral genotypes prevalent in India.

Survival of HAV and HEV in the Soil Samples

Of the 403 samples collected from four locations of the Mutha river, 77 (19.1%) were positive for HAV and 20 (4.9%) were positive for HEV RNA (Table 1). Prevalence of HAV was significantly higher than that of HEV for all four locations ($p < 0.05$). The prevalence pattern was independent of the locations of sample collection. The higher prevalence of HAV at the P1 location (closest to the sewage treatment plant) emphasizes the implication of the sewage discharge in the Mutha river. The prevalence of HAV and HEV RNA (i.e. 19.1% and 4.9%, respectively) recorded in the present study is in concordance with our earlier reports on sewage [10] and river water [11] samples from the same city. It is interesting to note that the prevalence of HAV (76.56%) and HEV (25%) in the 64 river water samples collected from same locations and same time-points during our earlier study, were significantly higher than the prevalence in soil samples (p values for both <0.0001). Thus, for the last 10 years, HAV was more highly prevalent than HEV in the waste samples tested. All the control Pashan lake samples ($n = 57$) were negative for HAV and HEV, emphasizing the significant role of sewage discharge in maintaining a high prevalence of these enteric viruses.

Overall, at a constant temperature of 37°C, HAV was more stable than HEV, while under daily temperature variations, both viruses survive similarly. The highest and lowest temperatures in Pune recorded during the study were 40.5°C and 11.9°C, respectively. The rapid, earlier degradation of HEV, as compared with HAV, may be one of the factors in the lower endemicity of HEV [12].

Many studies have dealt with the prevalence of viruses in sewage samples and waste water samples [6,10,13,14] and survival of HAV under different conditions. These include the survival of HAV on fomites till 60 days [15], in bottled mineral water at 4°C for over 1 year and at room temperature for 300 days [16], and little or no decay in river water during an observation period of 48 days [17]. Incubation of

TABLE 1. Weekly HAV and HEV RNA levels in soil samples spiked with individual viruses (maintained at environmental temperature (ET) and 37°C) and its correlation with field positive samples

		ET				37°C		Field samples	
Month	Week	Average maximum (°C)	Average minimum (°C)	HAV RNA copies/mL	HEV RNA copies/mL	HAV RNA copies/mL	HEV RNA copies/mL	HAV positive	HEV positive
January	1	30.5	11.9	1.23×10^8	6.47×10^7	1.23×10^8	6.47×10^7	6	2
February	2	34.8	13.2	2.07×10^7	8.65×10^7	1.18×10^7	1.79×10^7	11	6
February	3	33.8	12.2	3.3×10^6	7.4×10^3	3.4×10^6	6.2×10^4		
February	4	32.7	12.0	1.8×10^6	4.0×10^3	2.7×10^6	5.3×10^4		
February	5	34.0	13.8	8.9×10^5	9.0×10^3	2.1×10^6	2.1×10^4		
March	6	35.5	14.7	5.3×10^5	5.1×10^3	1.2×10^6	1.18×10^4	8	–
March	7	37.4	14.3	2.2×10^5	4.8×10^3	5.2×10^4	2.07×10^3		
March	8	35.9	16.2	5.5×10^3	4.2×10^3	4.8×10^4	1.54×10^3		
March	9	35.5	17.1	Undetected	3.4×10^2	2.4×10^4	1.14×10^3		
April	10	36.7	18.4	Undetected	Undetected	1.8×10^4	9.6×10^2	5	–
April	11	38.9	20.1	Undetected	Undetected	1.2×10^4	Undetected		
April	12	39.1	20.7	Undetected	Undetected	1.7×10^4	Undetected		
April	13	38.6	21.9	Undetected	Undetected	7.6×10^3	Undetected		
May	14	40.5	22.0	Undetected	Undetected	Undetected	Undetected	8	4
June								13	6
July								–	–
August								6	–
September								9	–
October								6	–
November								5	2
December								–	–
Total								77	20

seawater from Spanish coastal areas with HAV for 30 days at 5°C and 25°C resulted in lower inactivation at 5°C and more pronounced decay at 25°C [18]. Though the detection methods are different, stability of HAV is noteworthy. The higher stability of HAV at a constant higher temperature than at fluctuating temperatures suggests that the virus survival/adaption is better at constant temperature in the incubator than at the fluctuating temperatures observed in nature. Differential HEV stability at both temperatures probably reflects the intrinsic property of the virus belonging to the Hepeviridae family. The changes in the soil parameters over a period of 1 year did not influence virus positivity. However, a larger series needs to be screened before definite conclusions are made.

In view of the long-term survival of these viruses in soil, the relative role played by the contaminated soil in the transmission of enteric viruses needs to be evaluated further. Definite measures should be taken to reduce HAV and HEV in water as well as soil, which could help in lowering the burden of HAV and HEV.

Author Contributions

V.A.A. and D.P. conceived and designed the experiments. D.P. and P.K. performed the experiments. D.P., P.K. and V.A.A. analysed the data. D.P. and V.A.A. wrote the paper. All authors read and approved the final manuscript.

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Transparency Declaration

The authors declare that there are no conflicts of interest in relation to the publication of this work.

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